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# Influences of Inoculation Methods and Phosphorus Levels on Nitrogen Fixation Attributes and Yield of Soybean (*Glycine max* L.) At Haru, Western Ethiopia

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## ABSTRACT

An experiment consisting of four inoculation methods and four phosphorus levels was conducted under field condition in western Ethiopia to identify appropriate inoculation method and optimum phosphorus level which enhance nitrogen fixation attributes and yield of Clark 63K soybean. None inoculation, seed, soil and seed+soil inoculation methods were combined with 0, 20, 40 and 60 kg P $ha^{-1}$ and were arranged in factorial RCBD design. The crop was assessed for nodule number, nodule volume, nodule dry weight, shoot nitrogen content, number of pod bearing branches, shoot dry matter, plant height, number of pod, pod length, number of seed per pod, above ground biomass, seed yield, hundred seed weight and harvest index. The result showed that interaction effect of the main factors significantly ($p<0.05$) influenced nodule volume, nodule dry weight, number of pod and shoot dry matter per plant as well as shoot nitrogen content. Inoculation method did not significantly ($p>0.05$) influence nodule volume, nodule dry weight, shoot dry matter and number of pod per plant as well as shoot nitrogen content when the soybean was grown without phosphorus. Seed, soil and seed+soil inoculations significantly ($p<0.05$) increased plant height, number of pod bearing branches per plant, pod length, number of seeds per pod, above ground biomass, seed yield over the check. Seed and seed+soil inoculations showed superiority to none inoculation and soil inoculation in plant height, shoot dry matter, number of pod per plant and seed yield. The three phosphorus levels (20, 40 and 60 kg P $ha^{-1}$) resulted in significantly ($p<0.05$) higher nodule number, nodule volume and nodule dry weight per plant as well as shoot nitrogen content than unfertilized check in seed, soil and seed+soil inoculations. Seed, soil and seed+soil inoculations gave 26.12, 15.07 and 27.92% seed yield advantage over uninoculated check in that order. Moreover, fertilization of 20, 40 and 60 kg P $ha^{-1}$ had 16.67, 42.50 and 51.20% yield advantage over unfertilized treatment. When the crop was grown without inoculation and soil inoculation, 20 kg P $ha^{-1}$ was enough for the soybean to accumulate higher shoot dry matter and number of pods per plant. However, for seed and seed+soil inoculations, 40 kg P $ha^{-1}$ was identified as optimum.

**Key words:** *Bradyrhizobium,* inoculation method, nodulation, phosphorus level, soybean, yield

## INTRODUCTION

Soybean (*Glycine max* L.) is the world's important food legume of great nutritional value. The crop has the highest protein content (40%) of all food crops and is equivalent to proteins of animal products. It is the second only to groundnut in terms of oil content (20%) among food legumes. Soybean obtains significant portion of its nitrogen requirement from symbiotic $N_2$ fixation when grown in association with effective and compatible *Bradyrhizobium* strains. However, soils may not contain these strains to establish an effective association and hence inoculation is essential to ensure presence of effective *Bradyrhizobium* population in rhizosphere of the crop (Fouilleux *et al*., 1996; Kyei-Boahen *et al*., 2002).

Nowadays, biological nitrogen fixation via legume-*Rhizobium* symbiosis is attracting considerable attention of researchers because it is economically viable nitrogen input for resource poor farmers and environmentally friendly (Bejiga, 2004; Hailemarim and Tsigie, 2006; Wolde-meskel, 2007; Ellafi *et al*., 2011). Appropriate method of *Rhizobium* application is necessary to boost biological nitrogen fixation. Studies on rhizobia application methods revealed that majority of rhizobia applied to seeds via conventional seed inoculation method die on the seed before seeding or shortly after placement in the soil due to exposure to seed treatment chemicals, seed coat toxins, dehydration or excessive heat (Kyei-Boahen *et al*., 2002; Deaker *et al*., 2004). It is a general fact that information on relative efficiency of different inoculation methods of legumes is rare in Ethiopia.

Despite the substantial amount of total phosphorus in tropical soils, phosphorus deficiency is one of the most important fertility problems in tropical agriculture (Nyemba, 1986; Mengel and Kirkiby, 1987; Mamo *et al*., 2002). Importance of phosphorus in biological nitrogen fixation is well understood as it is an energy driven process and its availability is affected by soil pH. Most of Ethiopian highland soils are deficient in available phosphorus. Study on inoculation methods and different rates of phosphorus on soybean have not yet been conducted in Ethiopia as much as needed. Even, there is very little information on inoculation methods of legumes. Therefore, this study was initiated with the following objectives:

- To identify appropriate inoculation method which enhances nitrogen fixation attributes and yield of soybean under different phosphorus levels in the field condition of Haru
- To identify an optimum phosphorus level for different inoculation methods that enhances nitrogen fixation attributes and yield of soybean in the field condition of Haru

## MATERIALS AND METHODS

Field experiment consisting of four inoculation methods and four phosphorus levels was conducted at Haru, western Ethiopia in 2010 main cropping season. Haru Agricultural Research Sub Center is located at 5° 59' 10.79" N and 35° 47' 56.64"E coordinates and found at an elevation of 1740 m above sea level. The Sub Center is found at a distance of 475 km from Addis Ababa in western direction. The average annual rain fall of the center is 1727 mm and the mean daily maximum and minimum temperature are 27.5 and 13°C, respectively. The soil type of the center is acrisols (Mikru and Tena, 2008) and dominant agricultural types in the area are arable cropping and semi forest coffee production.

**Soil sampling and analysis:** A field of unknown history of soybean cultivation and *Bradyrhizobium* inoculation was chosen and composite soil sample was taken from the upper 0.3 m of the experimental area before planting. Selected physical and chemical properties of the soil were analyzed using standard laboratory procedures. Soil texture was determined following Bouyoucos hydrometer method. Soil pH was measured in the supernatant suspension of 1: 2.5 soil:

Table 1: Selected physical and chemical properties of the experimental soil

| Physical and chemical properties | Value |
|---|---|
| Sand (%) | 61.00 |
| Clay (%) | 28.00 |
| Silt (%) | 11.00 |
| Textural class | Sandy clay loam |
| pH $H_2O$ | 5.40 |
| OC (%) | 2.53 |
| OM (%) | 4.37 |
| N (%) | 0.87 |
| Available P (mg $kg^{-1}$) | 0.21 |
| CEC (cmol (+) $kg^{-1}$) | 12.84 |

OM: Organic matter, OC: Organic carbon, CEC: Cation exchange capacity

Water mixture by pH meter. Soil organic carbon was determined by Walkley and Black method (Walkley and Black, 1934; FAO, 2008). Total nitrogen was determined by Kjeldahl procedure. Available phosphorus was determined by Bray II procedure (Bray and Kurtz, 1945; FAO, 2008) and CEC were determined by leaching the soil with neutral 1 N ammonium acetate (FAO, 2008). Table 1 presented selected physical and chemical properties of the experimental soil.

**Treatments:** Four inoculation methods and four phosphorus levels were combined into sixteen treatment combinations and arranged in Randomized Complete Block Design (RCBD). Treatments were replicated four times. The inoculation methods used were no inoculation, seed inoculation, soil inoculation and seed+soil inoculation. Phosphorus levels used in the experiment were 0, 20, 40 and 60 kg P $ha^{-1}$. Non-inoculated and non-fertilized treatment was a check for the experiment. Factorial combinations of the factors into sixteen treatments were:

- No inoculation+0 kg P $ha^{-1}$
- No inoculation+20 kg P $ha^{-1}$
- No inoculation+40 kg P $ha^{-1}$
- No inoculation+60 kg P $ha^{-1}$
- Seed inoculation+0 kg P $ha^{-1}$
- Seed inoculation+20 kg P $ha^{-1}$
- Seed inoculation+40 kg P $ha^{-1}$
- Seed inoculation+60 kg P $ha^{-1}$
- Soil inoculation+0 kg P $ha^{-1}$
- Soil inoculation+20 kg P $ha^{-1}$
- Soil inoculation+40 kg P $ha^{-1}$
- Soil inoculation+60 kg P $ha^{-1}$
- Seed+Soil inoculation+0 kg P $ha^{-1}$
- Seed+Soil inoculation+20 kg P $ha^{-1}$
- Seed+Soil inoculation+40 kg P $ha^{-1}$
- Seed+Soil inoculation+60 kg P $ha^{-1}$

TAL 379, a commercial effective *Bradyrhizobium* strain was used as a source of inoculums whereas Triple Supper Phosphate was used as phosphorus source. Clark 63 K, a well adapted soybean cultivar in the area, was used as a test crop.

**Experimental procedures:** A field of unknown history of soybean cultivation and *Bradyrhizobium* inoculation was chosen and an area of 566.1 $m^{-2}$ was prepared. It was then divided into four blocks and every block was divided into sixteen plots of 7.2 $m^{-2}$ in size making a total of sixty four experimental units. Fine seed beds were prepared by leaving 1.0 and 0.5 m paths between blocks and plots, respectively.

For seed inoculation, soybean seeds were washed by tap water and surface sterilized with 70% ethanol. Seeds were then rinsed 3 to 4 times in sterilized tap water, moistened in sugar solution and inoculated by covering them with paste of inoculum which was made from a rate of 10 g of peat-based powder inocula per 100 g of seed just before planting (Somasegaran and Hoben, 1985). For soil inoculation, moist soil was taken from its own plot into a sterilized bucket and *Bradyrhizobium* were added to the soil with a ratio of 10 g: 1 kg (peat-based inocula: Soil) and thoroughly mixed. The mixture was placed under seeds. For simultaneous inoculation of seed and soil, both inoculations were applied. Phosphorus fertilizer, triple super phosphate, was applied to the plots as per the treatment and mixed with the soil evenly on the date of seed planting just before sowing. Finally, seeds were sown using 0.6×0.05 m spacing between rows and seeds, respectively. Uniform agronomic managements were applied for all plots.

**Data collection:** Number of pod bearing branches and shoot dry weight per plant were recorded from five representative plants at mid flowering. Nodule number, nodule volume and nodule dry weights per plant were also recorded from five representative plants. Nodule number was obtained by counting whereas nodule volume was determined by displaced volume. Nodule dry weight was obtained by weighing oven dried nodules, usually at 70°C for 1440 min, of five representative plants separately. The mean values of five plants were used for analysis for all of the above parameters. Then tissue nitrogen content was analyzed according to Kjeldahl procedure (FAO, 2008).

Plant height, pods per plant and seeds per pod were taken from five representative plants at maturity. Sub samples of five pods were taken from each of the five sampled plants and assessed for pod length as well as number of seeds per pod and the mean values were computed. Above ground biomass yield and seed yield were recorded per plot basis. The seed yield of the crop was then adjusted to 10% moisture content. Harvest index was computed by dividing seed yield to above ground biomass yield and hundred seed weight was recorded from three samples of seed lots after adjusting to 10% moisture content and averaged.

**Statistical analysis:** The data were subjected to analysis of variance by using the General Linear Model procedure of Statistical Analysis System (SAS) and Least Significant Difference (LSD) method at 0.05 probability level was used for mean separation. Count data such as nodule number, pods per plant, seeds per pod and pod bearing branches per plant were transformed by Square Root Transformation before analysis (Gomez and Gomez, 1984).

## RESULT AND DISCUSSION

### Influence of inoculation method and phosphorus level on nitrogen fixation attributes and shoot nitrogen content

**Number of nodule per plant:** Inoculation method significantly affected nodule number per plant (Table 2). Mean number of nodule in soil inoculated treatment was significantly ($p<0.05$) inferior to those obtained from seed and seed+soil inoculations. However, it was significantly more than the

Table 2: Influence of inoculation method and phosphorus level on nodule number $plant^{-1}$ of soybean at Haru

| Inoculation method | No. of nodules per plant |
|---|---|
| No inoculation | 15[d] |
| Seed inoculation | 34[b] |
| Soil inoculation | 31[c] |
| Seed+soil inoculation | 38[a] |
| $LSD_{0.05}$ | 2.40 |
| Phosphorus level (kg $ha^{-1}$) | |
| 0 kg P $ha^{-1}$ | 23[c] |
| 20 kg P $ha^{-1}$ | 30[b] |
| 40 kg P $ha^{-1}$ | 33[a] |
| 60 kg P $ha^{-1}$ | 31[ab] |
| $LSD_{0.05}$ | 2.40 |
| CV (%) | 11.50 |

Values (means) with different letters differ significantly at 0.05 probability level

number of nodules observed in uninoculated check (Table 2). Although, number of nodules per plant in an uninoculated treatment was significantly inferior to seed, soil and seed+soil inoculations, few nodules were observed on some soybeans. This might be due to the existence of naturalized indigenous *Bradyrhizobium* in that soil.

Phosphorus levels significantly affected nodule number per plant though its interaction with inoculation method did not (Table 2). Fertilizations of 20, 40 and 60 P kg $ha^{-1}$ increased nodule number per plant by 26.54, 41.93 and 34.10%, respectively, over the control. Number of nodules in the control was significantly inferior to those nodules obtained under other phosphorus levels. It was shown by pre planting soil analysis that available phosphorus in the experimental soil was so low and hence significant response was observed even when 20 kg P $ha^{-1}$ was applied.

**Nodule volume per plant:** Nodule volume was not significantly affected by inoculation method when phosphorus was not applied. However, seed, soil and seed+soil inoculations significantly ($p<0.05$) increased nodule volume over the uninoculated check under 20, 40 and 60 kg P $ha^{-1}$. Seed, soil and seed+soil inoculations did not give significantly different nodule volume except under 40 kg P $ha^{-1}$ where soil inoculation resulted in significantly lower nodule volume per plant than simultaneous inoculation of seed and soil (Table 3). The result indicated that 20, 40 and 60 kg P $ha^{-1}$ significantly ($p<0.05$) increased nodule volume over the control under seed, soil and seed+soil inoculations.

**Nodule dry weight per plant:** There was no significant effect of inoculation method on nodule dry weight when the soybean was not fertilized (Table 3). However, under applications of 20, 40 and 60 kg P $ha^{-1}$, seed, soil and seed+soil inoculations significantly ($p<0.05$) increased nodule dry weight over the uninoculated check. When the crop was grown in 20 and 40 kg P $ha^{-1}$, simultaneous inoculation of seed and soil significantly ($p<0.05$) improved nodule dry weight over their individual inoculation (Table 3). Nodule dry weight generally increased with phosphorus level in all inoculation methods (Table 3). However, nodule dry weights recorded from 20, 40 and 60 kg P $ha^{-1}$ supplied soybean were not significantly different when inoculation was not employed. Moreover, nodule dry weight of the 20 and 40 kg P $ha^{-1}$ supplied soybean was not significantly different under all inoculation methods. On the other hand, nodule dry weight of a 60 kg P $ha^{-1}$

Table 3: Interaction effect of inoculation method and phosphorus level on nodule volume per plant, nodule dry weight per plant and shoot nitrogen content of soybean at Haru

| | | Phosphorus level (kg P ha$^{-1}$) | | | |
|---|---|---|---|---|---|
| Parameter | Inoculation method | 0 | 20 | 40 | 60 |
| Nodule volume per plant (mL plant$^{-1}$) | No inoculation | $0.95^{g}$ | $1.13^{gf}$ | $1.15^{gf}$ | $1.20^{f}$ |
| | Seed inoculation | $1.10^{fg}$ | $1.83^{cde}$ | $1.90^{c}$ | $2.25^{ab}$ |
| | Soil inoculation | $1.08^{fg}$ | $1.78^{de}$ | $1.80^{d}$ | $2.23^{ab}$ |
| | Seed+soil inoculation | $1.05^{fg}$ | $1.98^{cde}$ | $2.05^{bc}$ | $2.30^{a}$ |
| | $LSD_{0.05}$ | 0.224 | | | |
| Nodule dry weight per plant (g plant$^{-1}$) | No inoculation | $0.16^{d}$ | $0.25^{c}$ | $0.26^{c}$ | $0.25^{c}$ |
| | Seed inoculation | $0.19^{d}$ | $0.36^{b}$ | $0.38^{b}$ | $0.41^{a}$ |
| | Soil inoculation | $0.18^{d}$ | $0.32^{b}$ | $0.35^{b}$ | $0.45^{a}$ |
| | Seed+soil inoculation | $0.17^{d}$ | $0.38^{b}$ | $0.41^{a}$ | $0.43^{a}$ |
| | $LSD_{0.05}$ | 0.054 | | | |
| Shoot nitrogen content (%) | No inoculation | $1.53^{h}$ | $3.21^{fg}$ | $3.40^{ef}$ | $4.00^{de}$ |
| | Seed inoculation | $1.89^{hg}$ | $4.11^{c}$ | $4.71^{bc}$ | $5.41^{ab}$ |
| | Soil inoculation | $1.21^{h}$ | $4.14^{cd}$ | $4.41^{cd}$ | $5.10^{b}$ |
| | Seed+soil inoculation | $1.79^{hg}$ | $4.70^{bc}$ | $5.33^{ab}$ | $5.80^{a}$ |
| | $LSD_{0.05}$ | 0.69 | | | |

Means followed by the same letter for a parameter are not significantly different at 0.05 probability level

supplied soybean was significantly superior to those recorded from 20 and 40 kg P ha$^{-1}$ fertilized soybeans when soil and seed+soil inoculations were employed.

**Shoot nitrogen content:** Inoculation method did not influence shoot nitrogen content of soybean when the crop was grown without phosphorus. However, seed, soil and seed+soil inoculations significantly increased shoot nitrogen content over the uninoculated check when the crop was grown in 20, 40 and 60 kg P ha$^{-1}$ (Table 3). Seed, soil and seed+soil inoculations did not give significantly different shoot nitrogen content when soybean was supplied with 20 kg P ha$^{-1}$ whereas seed and seed+soil inoculations resulted in statistically at par under 40 and 60 kg P ha$^{-1}$. Response of shoot nitrogen content of the soybean to phosphorus levels followed similar trend when the crop was grown without inoculation, seed or seed+soil inoculation. In this line, growing of the crop in 20, 40 and 60 kg P ha$^{-1}$ significantly increased shoot nitrogen content over the unfertilized control (Table 3). Variation of shoot nitrogen content of soybean was also reported by Asaminew (2007) and Zewdu (2009).

**Influence of inoculation method and phosphorus level on growth, yield and yield component**

**Shoot dry matter per plant:** Shoot dry matter per plant was not significantly ($p>0.05$) affected by inoculation methods under unfertilized check and 20 kg P ha$^{-1}$. Although, seed and seed+soil inoculations did not give significantly different shoot dry matter, they significantly ($p<0.05$) increased over soil inoculation. Within all inoculation methods, the shoot dry matter yield increased consistently with phosphorus level except in non inoculated soybean. Shoot dry matter per plant of 20, 40 and 60 kg P ha$^{-1}$ supplied soybeans was significantly ($p<0.05$) greater than those recorded from soybean grown without phosphorus under uninoculated, seed and soil inoculations. However, shoot dry matter per plant recorded from soybeans grown in these phosphorus levels was not

Table 4: Interaction effect of inoculation method and phosphorus level on shoot dry matter (g plant$^{-1}$) and number of pods per plant of soybean at Haru

| | | Phosphorus level (kg ha$^{-1}$) | | | |
|---|---|---|---|---|---|
| Parameter | Inoculation method | 0 | 20 | 40 | 60 |
| Shoot dry matter (g plant$^{-1}$) | No inoculation | $8^{ef}$ | $16^{bcd}$ | $17^{bc}$ | $15^{cd}$ |
| | Seed inoculation | $10^{e}$ | $14^{cd}$ | $19^{b}$ | $24^{a}$ |
| | Soil inoculation | $10^{e}$ | $15^{cd}$ | $15^{cd}$ | $18^{bc}$ |
| | Seed+soil inoculation | $10^{e}$ | $13^{de}$ | $19^{b}$ | $23^{a}$ |
| | LSD $_{0.05}$ | 3.79 | | | |
| No. of pods per plant | No inoculation | $17.80^{g}$ | $23.20^{ef}$ | $26.65^{cde}$ | $25.30^{b}$ |
| | Seed inoculation | $20.35^{fg}$ | $25.00^{de}$ | $32.40^{ab}$ | $34.85^{a}$ |
| | Soil inoculation | $21.08^{fg}$ | $27.45^{cd}$ | $29.60^{bc}$ | $31.15^{b}$ |
| | Seed+soil inoculation | $20.30^{fg}$ | $24.90^{de}$ | $30.80^{b}$ | $34.90^{a}$ |
| | LSD $_{0.05}$ | 3.50 | | | |

Means followed by the same letter for a parameter are not significantly different at 0.05 probability level

significantly different under uninoculated check and soil inoculation method (Table 4). Under seed and seed+soil inoculations, fertilization of 40 and 60 kg P ha$^{-1}$ significantly increased shoot dry matter per plant over 20 kg P ha$^{-1}$ (Table 4). This indicates that omission of P from optimum nutrition reduces dry matter yield of crop plants (Mengel and Kirkiby, 1987; Tena and Beyene, 2011).

**Number of pods per plant:** When the soybean was grown without phosphorus, their pod number was not significantly affected by inoculation method. Seed and seed+soil inoculations significantly ($p<0.05$) increased pod number per plant over the uninoculated check whereas soil inoculation resulted in statistically at par with all inoculation methods including the check when 40 kg P ha$^{-1}$ was applied. Under the highest phosphorus level (60 kg P ha$^{-1}$), number of pod per plant of soybeans grown in seed, soil and seed + soil inoculations was significantly ($p<0.05$) more than those recorded in uninoculated check. This result is in agreement with reports of Bhuiyan *et al.* (2008) and Malik *et al.* (2006) who concluded that pod per plant of mung bean and soybean is significantly increased by inoculating with *Bradyrhizobium*.

Supplying the soybean with 20, 40 and 60 kg P ha$^{-1}$ significantly increased number of pod per plant over the control (Table 4). Nevertheless, number of pod per plant produced by these levels was not significantly ($p>0.05$) different under non-inoculated and soil inoculated treatments. Under seed inoculation method, number of pod per plant of 40 and 60 kg P ha$^{-1}$ supplied soybeans was statistically at par, whereas the latter level significantly increased over the former under seed+soil inoculation.

**Plant height:** Inoculation method significantly influenced soybean height (Table 5). Soybean plants that were grown by seed and seed+soil inoculations were significantly taller than that were grown by soil inoculation. Soybean height in seed and seed+soil inoculations was not significantly different. Similar findings were also reported by Malik *et al.* (2006) and Shahid *et al.* (2009) who concluded that soybean height is significantly increased when inoculated with *Bradyrhizobium*. Height of the crop was correlated with phosphorus level (0.94*). All phosphorus levels resulted in significantly ($p<0.05$) taller soybeans than the check. Soybeans grown in 40 and 60 kg P ha$^{-1}$ were

Table 5: Influence of inoculation method and phosphorus level on growth, yield and yield components of soybean at Haru

| | Parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Plant height (m) | No. of pod bearing branches plant$^{-1}$ | Pod length (cm) | No. of seeds pod$^{-1}$ | Above ground biomass (kg ha$^{-1}$) | seed yield (Kg ha$^{-1}$) | HSWt (g) | HI |
| **Inoculation method** | | | | | | | | |
| No inoculation | 0.47$^{c}$ | 3.48$^{c}$ | 4.21$^{b}$ | 2.15$^{b}$ | 4740$^{c}$ | 1719$^{c}$ | 13.78 | 0.364$^{a}$ |
| Seed inoculation | 0.55$^{a}$ | 4.35$^{a}$ | 4.64$^{a}$ | 2.41$^{a}$ | 5990$^{b}$ | 2168$^{a}$ | 14.19 | 0.366$^{a}$ |
| Soil inoculation | 0.49$^{b}$ | 3.93$^{b}$ | 4.53$^{a}$ | 2.35$^{a}$ | 5740$^{b}$ | 1978$^{b}$ | 13.87 | 0.352$^{ab}$ |
| Seed+soil inoculation | 0.53$^{a}$ | 4.25$^{ab}$ | 4.68$^{a}$ | 2.45$^{a}$ | 6890$^{a}$ | 2199$^{a}$ | 14.34 | 0.327$^{b}$ |
| LSD $_{0.05}$ | 0.23 | 0.40 | 0.18 | 0.19 | 630 | 138 | ns | 0.028 |
| **Phosphorus level** | | | | | | | | |
| 0 kg P ha$^{-1}$ | 0.45$^{c}$ | 3.0$^{c}$ | 4.04$^{c}$ | 1.95$^{c}$ | 4350$^{c}$ | 1584$^{c}$ | 13.31$^{c}$ | 0.371 |
| 20 kg P ha$^{-1}$ | 0.49$^{b}$ | 4.0$^{b}$ | 4.42$^{b}$ | 2.24$^{b}$ | 5410$^{b}$ | 1848$^{b}$ | 13.80$^{bc}$ | 0.346 |
| 40 kg P ha$^{-1}$ | 0.54$^{a}$ | 5.0$^{a}$ | 4.73$^{a}$ | 2.56$^{a}$ | 6540$^{a}$ | 2258$^{a}$ | 14.31$^{ab}$ | 0.347 |
| 60 kg P ha$^{-1}$ | 0.54$^{a}$ | 5.0$^{a}$ | 4.87$^{a}$ | 2.61$^{a}$ | 7080$^{a}$ | 2395$^{a}$ | 14.76$^{a}$ | 0.345 |
| LSD $_{0.05}$ | 0.23 | 0.40 | 0.18 | 0.19 | 630 | 138 | 0.75 | ns |
| CV (%) | 6.28 | 14.11 | 5.60 | 11.18 | 15.03 | 9.61 | 7.50 | 11.28 |

Means with in a column with the same letter are not significantly different at 0.05 probability level, ns: Means are not significantly different at 0.05 probability level, HSWt: Hundred seed weight, HI means harvest index

significantly taller than those grown in 20 kg P ha$^{-1}$. Similar, findings were reported by Shahid *et al.* (2009) on soybean.

**Number of pod bearing branches per plant:** Seed, soil and seed+soil inoculations significantly increased number of pod bearing branches per plant compared with the control (Table 5). Number of pod bearing branches in seed inoculation was significantly ($p<0.05$) more than soil inoculation. Pod bearing branches obtained from simultaneous inoculation of seed and soil was not significantly different from those obtained under individual inoculation of seed and soil. This result is in agreement with a report of Shahid *et al.* (2009) on soybean. Phosphorus level significantly affected number of pod bearing branches though its interaction with inoculation method did not (Table 5). It was highly correlated with phosphorus level ($r = 0.96^{**}$). Number of pod bearing branches of 20, 40 and 60 kg P ha$^{-1}$ supplied soybeans was significantly higher than those observed from unfertilized ones. Similar findings were reported by Shahid *et al.* (2009).

**Pod length:** Seed, soil and seed+soil inoculations did not give significantly ($p>0.05$) different pod length though they gave significantly ($p<0.05$) longer pods than the control. This might be due to higher nitrogen content of the experimental soil. Significant increase of soybean pod length has also been reported by Shahid *et al.* (2009) when seed of the crop is inoculated. Pod length was highly correlated with phosphorus level ($r = 0.98^{**}$). Pod length recorded in 40 and 60 kg P ha$^{-1}$ was not significantly different ($p>0.05$) but significantly longer than that of the 20 kg P ha$^{-1}$ fertilized soybeans (Table 5). Significant effect of phosphorus level was also reported by Ogunlela *et al.* ( 2012) on Lablab.

**Number of seeds per pod:** Seed, soil and seed+soil inoculations significantly improved seeds per pod over the uninoculated check (Table 5). This result is in agreement with reports of

Bhuiyan *et al*. (2008) and Shahid *et al*. (2009) who concluded that seed per pod of inoculated mung bean and soybean was significantly improved over uninoculated treatment. However, seeds per pod recorded in seed, soil and seed+soil inoculations were not significantly different ($p>0.05$). It was shown that number of seed per pod was highly correlated with phosphorus level ($r = 0.96^{**}$). Number of seed per pod of 20, 40 and 60 kg P ha$^{-1}$ fertilized soybeans was significantly ($p<0.05$) more than the unfertilized check. On the other hand, number of seed per pod of 40 and 60 kg P ha$^{-1}$ supplied soybeans was not significantly different but significantly more than that of 20 kg P ha$^{-1}$. This result is in line with the findings of Shahid *et al*. (2009) and Malik *et al*. (2006) who reported significant increase of seed per pod of soybean by phosphorus fertilization.

**Above ground biomass:** Inoculation method and phosphorus level significantly influenced above ground biomass but their interaction effect did not (Table 5). Above ground biomass obtained in individual inoculation of seed and soil was not significantly different ($p<0.05$). Seed, soil and seed+soil inoculations increased above ground biomass by 26.37, 21.09 and 45.36%, respectively, over the check. Above ground biomass of the soybean was highly correlated with phosphorus level ($r = 0.98^{**}$). Application of either 40 or 60 kg P ha$^{-1}$ significantly increased biomass of soybean over the control and 20 kg P ha$^{-1}$ though they did give statistically at par (Table 5). Investigation by Malik *et al*. (2006) is in agreement with this report. The result revealed that fertilization of soybean with 20, 40 and 60 kg P ha$^{-1}$ increased above ground biomass by 24.36, 50.34 and 62.76%, respectively.

**Seed yield:** Inoculation method significantly influenced seed yield of soybean though its interaction with phosphorus did not (Table 5). Seed, soil and seed+soil inoculations significantly ($p<0.05$) improved seed yield over the uninoculated check. Seed yield obtained from seed and seed+soil inoculations was not significantly different ($p>0.05$). Similar finding was reported by Ahmed *et al*. (2008) on lentil. Seed yield increase by seed, soil and seed+soil inoculations over the check were 26.12, 15.07 and 27.92%, respectively. Seed yield of the soybean was highly correlated with phosphorus level ($r = 0.98^{**}$). Seed yields of 40 and 60 kg P ha$^{-1}$ supplied soybeans were statistically at par but significantly higher than that of 20 kg P ha$^{-1}$ (Table 5). Similar findings were reported on soybean where inoculation and phosphorus application significantly increased seed yield (Kumaga and Ofori, 2004; Malik *et al*., 2006; Fatima *et al*., 2007; Shahid *et al*., 2009). Fertilization of 20, 40 and 60 kg P ha$^{-1}$ had 16.67, 42.50 and 51.20% yield advantage over the control, respectively.

**Hundred seed weight:** Hundred seed weight of the soybean was not significantly affected by inoculation method (Table 5). This outcome is in contrast to the findings of Bhuiyan *et al*. (2008) and Ahmed *et al*. (2008). This might be due to high nitrogen content of the experimental soil (Salvagiotti, 2008). Hundred seed weight of the crop was very highly correlated with phosphorus ($r = 0.99^{***}$). Significant influence of phosphorus on hundred seed weight of soybean was also reported by Shahid *et al*. (2009).

**Harvest index (HI):** Harvest index recorded in seed inoculated and uninoculated treatments was significantly ($p<0.05$) higher than soil and seed+soil inoculations. Phosphorus level had no influence on harvest index of the soybean under investigation (Table 5).

## CONCLUSION

Inoculation is not necessary when phosphorus is not applied. When Clark 63K soybean is grown with seed and seed+soil inoculations, a phosphorus level of 40 kg P $ha^{-1}$ is identified as an optimum. However, 20 kg P $ha^{-1}$ is enough for the crop when it is to be grown without inoculation and by soil inoculation.

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